Fundamental Study of Nano-Scale Transporting System Utilizing Kinesin-driven Microtubules

著者	杉田 修啓
number	1
学位授与機関	Tohoku University
学位授与番号	医工博第1号
URL	http://hdl.handle.net/10097/51235

氏名（本籍地）	すぎた しゅうけい 杉田 修啓
学位の種類	博 士（医工学）
学位記番号	医工博 第 1 号
学位授与年月日	平成２０年 9月 3日
学位授与の要件	学位規則第４条第１項該当
研究科、専攻	東北大学大学院医工学研究科（博士課程）医工学専攻
学位論文題目	Fundamental Study of Nano-Scale Transporting System Utilizing Kinesin-driven Microtubules（キネシン駆動微小管を利用したナノスケール輸送システムに関する基礎研究）
論文審査委員	（主査）東北大学教 授 佐藤 正明 東北大学教 授 田中 真美　東北大学教 授 西澤 松彦（工学研究科） 東北大学教 授 橋本 浩一（情報科学研究科）

論文内容の要旨

Chapter 1. Introduction

Kinesins, biomolecular motors that move along microtubules (MTs) in cells, can potentially be utilized as nano-scale transport systems because the motors have nano-scale size. Previous *in vitro* studies have shown that the motility of the motor proteins was reconstituted in an inverted gliding geometry, in which MTs glide on a kinesin-coated surface. For developing the transport systems with this geometry, key requirements were precise controls of motility such as gliding direction and gliding velocity of MTs. This study aims to establish the fundamental technique for control of MT motility using electric field and topographically structured substrate.

Chapter 2. Effects of Microtubule Length and Kinesin Density on their Motility

In this chapter, angular velocity and gliding velocity of MTs were quantitatively measured, particularly focusing on the length of MTs and kinesin density to understand the details of motility properties of MTs. In this study, experiments were performed with an in vitro gliding assay system. Kinesins were expressed in *E. Coli* and was purified, and MTs were polymerized with paclitaxel at 37°C. A flow cell with straight channel was fabricated by sandwiching the spacers with substrate such as a coverslip, and *in vitro* gliding assay system was constructed in the channel by coating the kinesin on the inner surface of the ehannel via protein complex of biotinylated albumin and streptavidin. Then, gliding MTs on the kinesin-coated surface were observed in the assay buffer including the adenosine triphosphate with an inverted fluorescent microscope. The angular velocities of the gliding MTs originally being due to thermal diffusion significantly decrease with increasing of the length of MTs for the low (7.5 μg/ml) and middle (38 μg/ml) kinesin densities although the decrease is not significant for the high (75 μg/ml) kinesin density. This result suggests that both the number of kinesins associated with MTs and the spacing between neighboring kinesins may contribute to the gliding direction. In contrast, the gliding velocity is not significantly affected by the MT length, ranging from 0.3 to 0.5 μm/s on average with decreasing the kinesin density. This may potentially imply the existence of an underlying mechanism with respect to the number of kinesins per the unit length of MTs. Towards development of high throughput nano-scale transport systems, these results reveal that long MTs associated with many kinesins and low densities of kinesin on the surface would be effective for producing high directionality and high velocity, respectively.

Chapter 3. Size-Sorting of Microtubules with Channels

To select the MTs appropriate for the carriers, the micro-scale channels were fabricated on a chip and phenomena of kinesin-driven MTs approaching to the channel were analyzed. As a preliminary experiment, the density of attaching MTs and their gliding velocity were investigated on silicon, gold, and glass substrates to select the appropriate substrate for fabrication of the micro-channels. As a result, the density of attaching MTs and gliding velocity on gold were high and comparable to that on the glass, which is normally used as a substrate for the gliding assay. Taking together with convenience of fabrication, silicon substrate coated with gold layer was selected. Observation of the gliding MTs over the channels showed five different phenomena; bridging, guiding, detaching, stopping, and falling. The probabilities for occurrence of these phenomena depended on the parameters of the incident angle of the MT approaching to the channel, the magnitude relation between the MT length and the channel width, and concentration of methylcellulose of the solution. For MTs longer than the channel width, the probabilities for bridging and guiding were high for the MTs approaching with the high and low incident angle, respectively. On the other hand, for MTs shorter than the channel width, the probabilities for detaching and stopping were high for the MTs approaching with high incident angle, whereas guiding was mainly observed for the MTs approaching with the low incident angle. It should be noted that the bridging never happened for the MTs shorter than the channel width. Furthermore, when MTs were approached to the less than 6 μm of channels with the 30°–60° of incident angle, MTs longer and shorter than the channel width showed more than 40% of bridging and more than 40% of guiding, respectively, in 0.2% of methylcellulose. By increasing the methylcellulose concentration up to 0.5%, this probability increased up to more than 80% of bridging and 60% of guiding. Since both the bridging and guiding show the high probability under the 30°–60° of incident angle in 0.2% of methylcellulose concentration, and the difference of the phenomena between bridging and guiding is determined by the magnitude relation between MT length and channel width, these results indicated that the feasibility for developing the device sorting the MTs by their length.

Chapter 4. Semi-Automated Tip-Tracking Algorithm for Gliding Microtubules

To control motility of the gliding MTs, detecting the position and the gliding direction of the MTs is required. In the gliding assays, the MTs are propelled along the surfaces by the kinesin molecules with the leading tip retraced by the rest of the MTs. Therefore, the author suggests an algorithm that precisely detects the trajectory by tracking the position of the MT leading tip. The algorithm operates on a stack of 150 fluorescent images of the gliding MTs with a 1 s interval and extracts the leading tip by using binarization, skeletonization, filtration with the 3x3 kernel with weight of 1 for all, and extracting the pixels with the intensity of 2. For verification of the algorithm, the algorithm was firstly applied to a sample segment with a certain given length, width, and curvature radius. The accuracy analysis of the algorithm showed that the changes in the length and curvature radius of the segments did not affect the difference from the given coordinates of the tips of the segments, whereas the changes in the width of the segments produced a clear difference (2 pixels in the size of MTs in fluorescent images). However the difference was negligible small and did not affect the tip tracking. The algorithm was then applied to the gliding MTs, resulting in good agreement in comparison of the tip tracking-based trajectory to the actual trajectory. The "leading tip" marker recognition algorithm may be useful for controlling the MTs dynamically.

Chapter 5. Fundamental Analysis of Directing Microtubules Utilizing Static Electric Fields

To understand the effect of EF on the gliding direction of MTs, the angular velocity of gliding MTs during electric field (EF) application was characterized. The buffer reservoirs were placed at the each end of the channel, and electrode was inserted into each reservoir. By providing the electrical potentials to the electrode, 10, 20, and 50 V/cm of EF were applied to the gliding MTs, and angular velocity of the gliding MT was analyzed. As a result, the angular velocity of gliding MT was in proportion to not only the EF strength but also the sine of the angle between the gliding direction of the MTs and the electric force direction. This result indicates that perpendicular component of the electric force to the gliding MTs affects the changes in the angular velocity. According to a previous report, we assumed a kinesin-driven MT applied with electric force as a beam supported by simple supports, overhanging from a support, and loaded by a distributed load, and the deflection angle of the MT tip was calculated. Electric force per unit MT length was calculated to be 1.6 pN/μm at the 50 V/cm from the measured the electrophoretic velocity of MTs, electroosmotic flow of the assay buffer, and viscosity of the assay buffer. The maximum length of the MT tip overhanging from a kinesin was estimated from kinesin spacing to be 1.8 μm which was obtained from the superimposing the sequential images of gliding MTs and analyzing the distance between the points that the MT always passed through. The electric force and estimated overhang length gave the theoretical curvature radius of 8.8 and 7.3 μm in 0.1% and 0.2% methylcellulose solution, respectively. These are in good agreement with the experimental curvature radius of 6.2 and 6.7 μm for EF strength of 50 V/cm in 0.1% and 0.2% methylcellulose solution, respectively. Hence, the changes in the gliding direction of MTs can be theoretically expressed with the deflection of a beam, indicating that the both electric force and spacing between kinesins were determinant factors for the curvature radius of the trajectory of gliding MTs. Although EF application is an efficient tool to control the gliding direction of MTs, MTs tended to detach from the surface with increasing of the EF strength in 0.1% methylcellulose concentration. We confirmed that the detaching was prevented in the 0.5% methylcellulose solution. However, since the angular velocity of gliding MTs decreased with the increasing of the methylcellulose concentration, the appropriate concentration was determined as 0.2% in this study. These results are useful for controlling the trajectories of gliding MTs quantitatively and precisely.

Chapter 6. Active Directional Control of Microtubules Utilizing Perpendicularly Applied Electric Fields

To control the gliding direction of the MTs in a desired trajectory, the dynamically changed direction of EF was applied to the gliding MTs. As an example of the dynamic control of the gliding direction, EF was perpendicularly applied to the gliding MTs, and rotated them with constant speed to revolve the MTs gliding on kinesins. The flow cell with the crossed channels was fabricated, and a buffer reservoir was placed at each end of the channel and an electrode was inserted into each reservoir. Before applying the EF, the distribution of the EF strength was calculated with a simulation model, and the magnitudes of the electrical potentials of electrodes were regulated to generate the about 50 V/cm of EF strength at the intersection of the channels. By changing the magnitude of the electrical potential of each electrode, EF in the intersection of the channels was generated in the desired direction. By applying 50 V/cm of EF rotated with constant speed from 1.4°/s to 3.3°/s to the crossed channels, some MTs showed circular loci, indicating the success of the dynamic control of gliding MTs by EF application. The probability of success in revolving of MTs was higher (> 40%) for rotating speeds of the EF of 2.4°/s or less, whereas it was quite low (< 10%) for rotating speeds of the EF of more than 2.4°/s. Since the maximum angular velocity of gliding MTs was 2.4°/s under the comparable environmental condition (50 V/cm of EF, 0.2% methylcellulose, 1.8 μm of spacing between kinesins), gliding MTs seemed not to follow the change of the direction of EF rotating at more than 2.4°/s. The curvature radius of revolving MTs of 8.3 μm at the rotating speed of EF of 2.4°/s was also comparable to the result of 6.7 μm obtained from the result of the fundamental motility property of MTs applied with the EF. The result was about a half of the curvature radius in previous reports, indicating the effectiveness of application of EF perpendicular to the gliding MTs. However, even in the case of EF rotating speed of 2.4°/s or less, some MTs did not show the circular locus. As the result of the analysis of gliding velocity during rotating EF application, the gliding velocities of MTs drastically decreased at the point that out of control trajectories of MTs initiated. For controlling the trajectory of gliding MTs more precisely, control of gliding velocity might be required.

Chapter 7. Specificity of Gliding Velocity of Microtubules to Direction of Electric Field

In this chapter, we characterized the changes in the gliding velocity of MTs for the case that EF was applied parallel to the gliding direction of MTs. After the gliding MTs were aligned with the preliminary EF application, 10, 20, 50, and 75 V/cm of EFs were applied parallel to gliding MTs so that the direction of electric force was same as (forward EF application) or opposite to (backward EF application) the gliding direction of MTs, and the gliding velocity of MTs was measured during EF application. The gliding velocity of the MTs increased with the increasing of EF strength for the forward EF application, whereas it decreased with the increasing of the EF strength for the backward EF application in dose-dependent manner. The response time required the changes in the gliding velocity was within 5–10 s after EF application. These results indicate that electrical control has feasibility for the dynamic control of the gliding velocity of MTs.

Chapter 8. Concluding Remarks

In this study, the author performed the fundamental analysis of motility properties of gliding MTs and controlled their motility with EF. The results obtained should be useful information for developing the nanoscale transport systems.

論文審査結果の要旨

キネシンは細胞骨格である微小管に沿って動く生体分子モータであり、細胞内において小胞等を運搬している。近年、細胞外においてキネシンコート表面上を動く微小管として滑り運動が再現され、分子モータをナノスケールでの輸送システム構築に利用することが提案されている。このシステム構築のためには、分子の滑り運動挙動を詳細に制御できることが課題であり、輸送担体を動的にかつ任意方向に制御することが必要である。本論文は、これらの研究成果をまとめたものであり、全編8章からなる。

第1章は序論であり、本研究の背景、目的および構成を述べている。

第2章では、微小管の長さおよびキネシンの表面密度の変化による運動速度および方向変化の基礎検討を行っている。低キネシン密度条件のみにて微小管の速度が微増し、長い微小管や高キネシン密度条件では微小管が直線的に運動する現象という結果を得ており、輸送システム構築の基礎的情報として有益な成果である。

第3章では､滑り運動する微小管が基板上に構築した溝に進入した際の挙動を詳細に解析している。微小管の溝への進入条件に依存して、微小管が到達する範囲が大きく異なる現象を発見し、これを利用した微小管のソーティングデバイスを提案している。これは、斬新なアイディアであり、非常に重要な成果である。

第4章では、微小管の運動制御に重要な運動先端を半自動的に抽出するアルゴリズムについて述べている。アルゴリズムの検証により微小管運動解析手法としての有効性を確認しており、輸送システムの実用化に向けた重要な成果である。

第5章では、輸送担体である微小管の運動方向を制御するため、微小管に電場を負荷した際の運動解析を行っている。電場負荷条件として滑り運動方向に対する電場負荷角度が重要であることを発見し、また運動軌跡が材料力学を利用した理論値と一致することを確認している。これは微小な輸送担体の運動挙動を詳細に制御するために重要な知見である。

第6章では、滑り運動する微小管に対する電場負荷方向を動的に制御し、微小管の運動方向の継続的な変化が可能なことを示している。微小管は事前に予測した曲率半径による移動経路とほぼ同一な経路上を滑り運動し、滑り運動方向を詳細に制御することが可能である。これは、輸送担体を動的かつ任意方向に制御する手法を示した重要な成果である。

第7章では、微小管の滑り運動方向と平行方向に電場を負荷して滑り運動速度への影響を検討し、負荷した電場による力の大きさに依存して微小管の滑り運動速度が変化することを示している。これは輸送担体の動的な速度制御として新たな手法を示した重要な成果である。

第8章は結論である。

以上要するに本論文は、ナノスケールでの輸送システムとしてタンパク質を利用し、そのシステムを構築するために必要な輸送担体の自由な運動制御が可能であることを示したものであり、医工学および機械工学の発展に寄与するところが少なくない。

よって、本論文は博士（医工学）の学位論文として合格と認める。